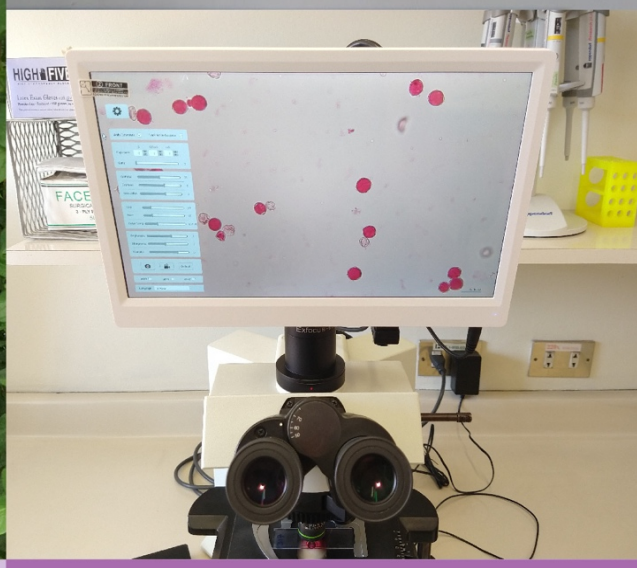

Guide for evaluation of potato essential reproductive characteristics in greenhouse conditions

International Potato Center (CIP)

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Guide for evaluation of potato essential reproductive characteristics in greenhouse conditions

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Guide for evaluation of potato essential reproductive characteristics in greenhouse conditions

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Content

1 Introduction

Reproductive biology in plants encompasses flowering and pollination, microsporogenesis, megasporogenesis, pre- and post-fertilization barriers, embryogenesis, and endosperm development. For potato breeding and evolution, the essential mechanisms for hybridization include: 2n gametes sexual polyploidization, endosperm balance number incompatibility system, and fertility barriers such as male sterility and S-alleles based incompatibility.

Conventional potato breeding starts by selecting and crossing parents with desirable complementary traits. Hence, understanding reproductive biology helps us to design precise crossing plans that will result in a successful hybrid progeny (Hanneman, 1999; Muthoni et al., 2014).

This guide lists protocols and procedures for the flow of breeding activities during the evaluation and screening of reproductive traits for any potato population. In this guide, we provide detailed information from greenhouse set up to planting and laboratory protocols, registry, and data analysis.

2 Review of literature

2.1 Flowering

In most cases, potato inflorescences have 7-15 flowers; however, some carry up to 30 flowers per inflorescence. Flowers have five sepals, five petals, and the corolla will form a star to rounded shape depending on the genotype. Flower color can vary from white to a complex range of blues and purples (Gómez, 2000; Muthoni et al., 2014). Five stamens are attached to the corolla tube, and the anthers are bright yellow with purple pigmentation in some species. Pale yellow or yellow-green anthers are a sign of male sterility. The bilobed stigma protrudes between the anthers (Gómez, 2000; Muthoni et al., 2014; Ordoñez et al. 2017).

Flowering behavior in potato is determined by the genotype, photoperiod, light intensity, and temperature. For early maturing genotypes flowering starts between 39 and 45 days after plantation (DAP), and its peak usually is at 60 DAP (Celis-Gamboa, 2002; De Haan et al., 2014). For late maturing genotypes, flowering peak can occur at 90-100 DAP. Flowering in potato is best under long days (~16 hours photoperiod) and night temperatures of 15–20 °C. Short days may lead to floral abscission (Almekinders and Struik, 1996).

Flowering and tuberization are two intertwined processes. Nevertheless, light spectrum and intensity, daily light integral (DLI), and photoperiod can be adjusted to induce one particular type of reproduction. According to Plantenga (2019), the appearance of flower buds is not affected by red light, blue light or day length, and high DLI values ($10 < DLI < 25$ mol·m^{-2}· d^{-1}) can accelerate flowering time under short and long days. Tuberization is delayed with blue light and long photoperiods.

Thus, a treatment for flowering induction can involve a long photoperiod, blue light, and increasing light intensity until reaching a DLI higher than 10 mol·m^{-2} d^{-1} (Plantenga, 2019).

2.2 Pollen production and 2n gametes.

Pollen is the product of plant microsporogenesis. These cells contain the haploid (n) genetic information for any plant genotype and are a fundamental piece within the breeding and evolution process. In general, vigorous and viable pollen will successfully germinate from the stigma to the ovary of the plant and fertilize an egg (n) to produce an embryo (2n). Potato pollen is known for producing unreduced gametes (2n) by two mechanisms with different genetic consequences: first division restitution (FDR) by parallel spindles and second division restitution (SDR) by premature cytokinesis. The outcomes are diploid gametes with different levels of heterozygosity; FDR 2n gametes have the highest level of heterozygosity (Mok and Peloquin, 1975). It is crucial to quantify the amount of viable 2n pollen because it is a medium for unilateral sexual polyploidization.

To maintain and evaluate pollen, it must be collected from mature anthers. Anther dehiscence occurs 3 -5 days after anthesis. Bright yellow anthers with brown tips signal mature anthers, which are good sources of pollen (Ordoñez et al., 2017). Pollen viability is extended when stored on cool and dry conditions. Viability can be maintained for a month if pollen is stored at 2.5^{o}C or a year at –24^{o}C (Blomquist and Lauer, 1962).

2.3 Reproductive barriers

Unlike wild potato relatives or particular landraces, advanced tetraploid clones do not flower profusely. When they do, they are often male sterile. This result may be unintentional as breeders may accidentally select against flowering and reproductive characteristics when selecting positively for tuber yield and plant vigor (Muthoni et al., 2014).

Any genetic blockage from bud initiation to seed set may be a cause of sterility. Among the typical intrinsic failures are no flower production, low or unviable pollen production, male sterility, and self-incompatibility (Muthoni et al., 2014).

Hybridization barriers in potato include other types of reproductive problems. Pre-zygotic barriers include pollen rejection (self-incompatibility, governed by S-alleles), and unilateral incompatibility, in which pollination works only one way, usually between a self-compatible female and self-incompatible male. Post-zygotic barriers are related to the parental genome balance in the hybrid endosperm or interploidy crosses (Tonosaki et al., 2016).

During the development of this protocol, the intrinsic and pre-zygotic types of failures will be observed and evaluated.

3 Greenhouse Set Up

3.1 Plant management

For flowering evaluation of potato clones, plants will be planted in triplicate. Plant management will be similar to the treatment of female progenitors on potato crossing plans (Ordoñez and Orrillo, 2012). Potato plants from a tuber or in-vitro will be sown in small pots (0.4 to 0.6 liters). Transplanting is performed when plant height reaches 15–20 cm. Plants are then placed on the top of pots with compressed soil (approximate volume: 3 liters) to expose the stolons and facilitate pruning (Figure 1). Plant density will be increased compared with crossing plans because the crossings will not be carried out and there is no risk of pollinating adjacent plants. A density of 8-11 plants per m^2 is recommended for flowering evaluations and 4-6 plants per m^2 for crossing plans (Figure 2).

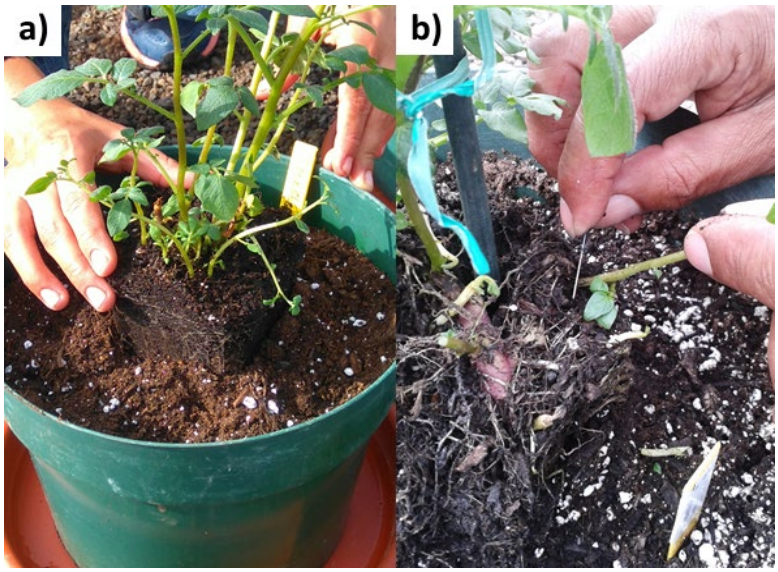


Figure 1. a) Transplanting of a parent on top of well-pressed soil. The stolons are exposed. b) Pruning stolons on the plant.

Figure 2. a) Crossing plan: Plants are placed in groups, leaving rows for easy management. More space allows larger plants and stronger trainers (e.g., bamboo canes) to be used. b) Flowering evaluation: Plants are set in continuous columns. In this layout, plants do not develop as strong as in crossing plans and smaller canes must be used as trainers. Lightbulbs provide necessary illumination (more information in section 3.2).

3.2 Lighting

Light is the most critical factor for potato development; its length and intensity can trigger tuberization or flowering. Long days ($\geq$ 16 hours daylight) inhibit tuber development by increasing the supply of assimilates to the apical shoots. For potato flower induction, a photoperiod of 16-20 hours is needed (Almekinders, 1995). Adding supplementary light from 01:00 to 06:00 by hanging white/blue lightbulbs (preferably LED) is effective for simulating long days. Lightbulbs are hung from the greenhouse ceiling to 30 cm above the plant, one lightbulb per m^2 should be used. The amount of daily light integral (DLI) should be equal or greater than 10 mol m^{-2} d^{-1}.

Potato plants are grown to induce flowering by hanging light bulbs from above as in Figure 2a. The cord of the lightbulb should be long enough to reach the plantlets after transplant. The cord will be rolled up as plants grow (Figure 2b). It is important to record and monitor the temperature, relative humidity and DLI during the experiment (Figure 3).

Figure 3. Sensors for DLI measurement (yellow) and for temperature and relative humidity (white).

3.3 Fertilization

Previous findings about fertilization doses for true potato seed (TPS) production are divergent (Roy et al., 2016; Roy et al. 2007; Zelalem et al., 1999; and Almekinders, 1995). Almekinders (1995) concluded that high rates of nitrogen during flowering does not have a significant effect on the number of flowers and inflorescences (45 kg/ha of nitrogen, four applications). Zelalem evaluated four doses of nitrogen and phosphorus separately in the range of 0-207 kg/ha and 0-60 kg/ha, respectively, and found that both levels promote vegetative growth in above and underground biomass and delayed days to flowering.

On the contrary, Suvra et al. (2007) tested ranges of doses of nitrogen of 0 - 300 kg/ha and doses of phosphorus of 0 - 180 kg/ha and found that increasing the concentration of both elements separately also increased the number of inflorescences and flowers per plant.

Comparing results of fertilization doses from the field and translating them to greenhouse pots is inconvenient. Thus, the formulation used at CIP for promoting flowering followed guidelines from tomato, where lower nitrogen doses and increased phosphorus and potassium are used to improve flowering and fruit yield (Kumar et al., 2013; Yara, 2019). For potato, doses can vary between genotypes. Usually advanced tetraploid cultivars require full doses (Table 1) while diploid clones and wild material require half doses.

Table 1. General doses and formulation of NPK fertilizers used during flowering and reproductive characteristics evaluation (half doses can be used on sensitive material).

Growing period	NPK ratio	Pot volume (l)	Dose (g l^{-1})	Volume applied (l)	Frequency (d)
0 – Transplant (30 - 45 DAP) *	None	0.5	None	None	None
Transplant - Flower initiation (~60 DAT)	21-7-7	3	3	0.3	10-12
Flower initiation – Maturing (~90 DAT)	10-30-20	3	5	0.3	10-12
Maturing – Harvest **	0-23-30	3	5	0.3	10-12

DAP: Days after planting; DAT: Days after transplanting.

* If presence of fungus (rhizoctonia or powdery mildew) is observed. Benomyl (Farmathe®) should be applied at a concentration of 1 g l^{-1}.

** A 1:1 mixed of Triple Super Phosphate 0 – 46 – 0 and Potassium chloride (KCl) 0–0– 60.

4 Evaluation of main flowering and cytological variables

Recording and registering evaluation data can be done via the Fieldbook App (Rife and Poland, 2014). The following variables should be considered.

4.1 Flowering degree (Flwdgr)

Flowering degree will be evaluated every 15 days starting at 45 days after transplanting (DAT). The number of evaluations will vary depending on the early or late nature of the genotypes to be evaluated. Having several evaluations over time is advised when there is uncertainty about the early or late development of a cultivar. This information will also allow us to determine the number of days for flowering initiation, peak of flowering, and senescence. For example, when working with early potato clones from advanced cycles of selection and homogeneous behaviour, the number of evaluations can be from 1 to 3. When working with late cultivars or wild potatoes with long vegetative cycles the number of evaluations can range 5 to 7.

Flowering degree will be evaluated categorically based on Gómez's guide (2000) for morphological characterization of native potato. Minor modifications are included in the description of the category (Table 2).

Table 2. Categories for evaluation of flowering degree and descriptions

Flowering degree category	Description
0	Absence of flower buds
1	Small inflorescences, flower buds or abscission of flower buds
3	Scarce flowering: 2-5 flowers per inflorescence
5	Moderate flowering: 6-12 flowers per inflorescence
7	Profuse flowering: >12 flowers/flower buds per inflorescence

Source: Gómez, 2000.

4.2 Predominant flower color (PFLC) and intensity (IPFLC)

Predominant flower color and intensity will also be evaluated categorically according Gómez's guide (2000) for the morphological characterization of native potato. This evaluation is performed on a recently open flower by observing the corolla and comparing its color with a color card (Figure 4). This information allows us to characterize a clone and and is taken once during the vegetative period due to its stability. Figure 4 shows a color card with eight predominant flower colors placed horizontally and three color intensities placed vertically. It is helpful to always do the comparative evaluations with the color card on hand (or uploaded in the Fieldbook app).

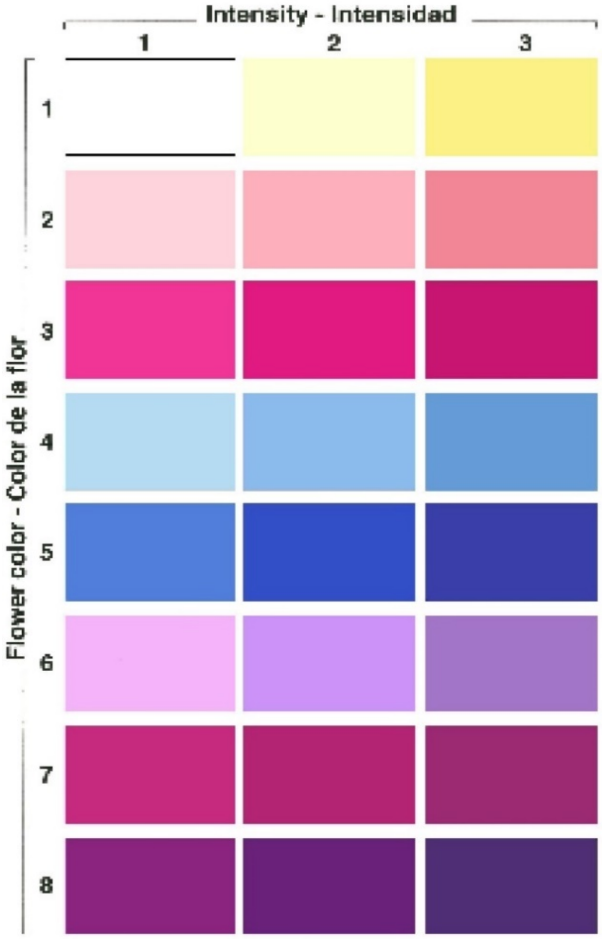


Figure 4. Color card for evaluating PFLC and IPFLC.

Source: Gómez, 2000.

4.3 Heterostyly (HSty)

The style is the central elongated structure of the pistil that connects the ovary to its stigma. Its polymorphism in length is called heterostyly. Heterostylous species can have two or more stylar morphs or categories of length. In potato, we found three morphs: styles shorter, equal to, or longer than the length of the stamens (Huamán, 1986; Wang et al., 2017). In this protocol, heterostyly is evaluated categorically (Table 3). Flowers from three inflorescences of the same plant will be observed and the predominant stylar morph will be rated. This evaluation is taken once. This trait has a practical utility for two reasons when designing crossing plans: 1) it is easier to cross long styles rather than short ones; and 2) when styles are short there is a higher probability of self-fertilization if the plant is self-compatible.

Table 3. Categories for visual estimation of the style length of the potato flower.

Heterostyly category	Description
1	Below stamens
2	Same height as stamens
3	Longer than stamens

4.4 Pollen quantity (QPol)

Pollen quantity will be measured categorically based on Salaman (1910) with some modifications. Pollen from ten flowers will be extracted and rated. The extraction method is performed according to CIP's manual of potato reproductive biology and cytology (Ordoñez et al., 2017). Anthers are collected on parchment paper and left to dry overnight at room temperature (~25 °C).. The next day pollen is collected by vibrating the anthers and emptying the pollen into a 500 µl Eppedorf® tube. The volume of the pollen can be rated according to categories in Table 4 and Figure 5.

Table 4. Categories for visual estimation of pollen quantity (QPol) and description.

QPol category	Volume (µl)	Description
0	Qpol = 0	Absence
1	0 < QPol ≤ 50	Little
3	50 < QPol ≤ 250	Moderate
5	250 < QPol	Abundant

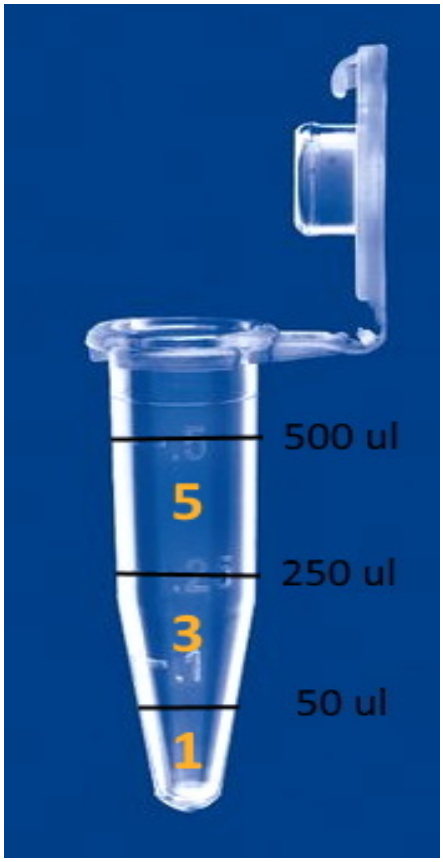


Figure 5. Reference lines for QPol rating using a 500 µl Eppendorf tube.

4.5 Pollen viability (PollV)

Pollen viability is rated according to its percentage of viability. To determine the percentage of viable pollen, we use the acetocarmine staining technique (Ordoñez et al., 2017). A small amount of pollen is

placed on a glass slide and stained with acetocarmine glycerol jelly and let to stain for 24 hours. The next day the slide is observed under a microscope at a 200x magnification. Well-shaped and stained pollen is considered viable: turgent, bright red with a homogenous cytoplasm. Unstained pollen, pink, or deformed grains are considered not viable (Figure 6). Count the colored pollen and the not colored pollen. A total count of 250 pollen grains across different regions of the slide is good to determine viability percentage. Use this equation:

$$\%viability = \frac{number\ of\ stained\ pollen\ grains}{total\ pollen\ grains} \times 100$$

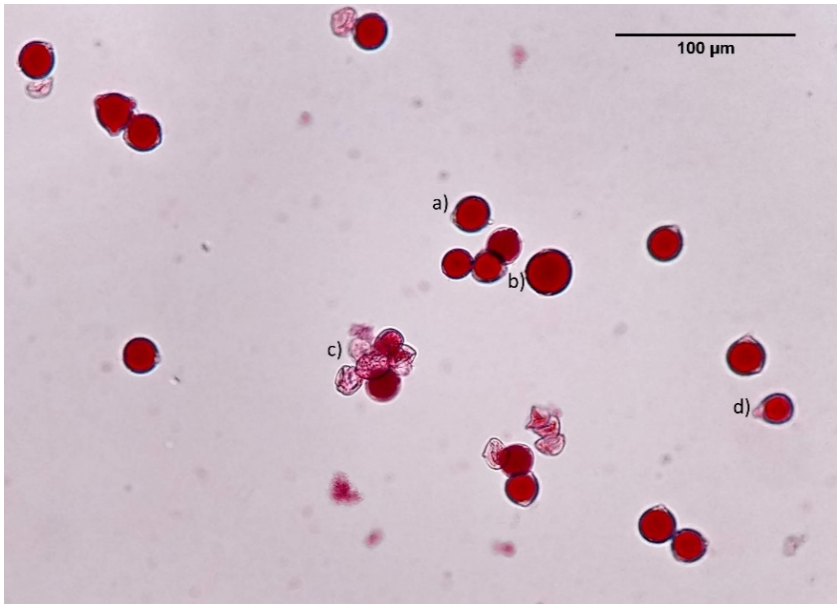


Figure 6. A sample of potato pollen stained with acetocarmine glycerol jelly. a) viable pollen, b) unreduced gamete, c) clump of unviable pollen, and d) pink pollen with uneven color, unviable.

Table 5. Categories for describing pollen viability (PollV)

Category	Viability range (%)	Description
1	0	Sterile
3	0 < % ≤ 50	Low
5	50 < % ≤ 80	Moderate
7	80 < % ≤ 100	High

Source: Ordoñez et al. (2017)

4.6 Unreduced gametes (2NPol)

Unreduced gametes will be evaluated simultaneously with pollen viability only in samples with a viability percentage above 60%. After evaluating pollen viability, on the same slide, switch the objective to a 1000x magnification and measure the diameter of 50 grains of pollen (If the microscope has an image capture device, then the measurements can be taken at 200x). Unreduced gametes (2n) can be detected by the bimodal distribution of their diameter and 2n pollen will have a diameter 1.2 times larger than reduced gametes (n) (Figure 7) (Ordoñez et al., 2017; Quinn et al., 1974). In Figure 8, we show an example of the data display. The percentage of 2n pollen is determined by this formula:

$$Percentage\ 2NPo = Frequency\ of\ 2N\ viable\ pollen\ x\ Pollen\ viability\ percentage$$

Figure 7. a) n pollen with a diameter of 15 µm; b) 2n pollen with a diameter of 21 µm.

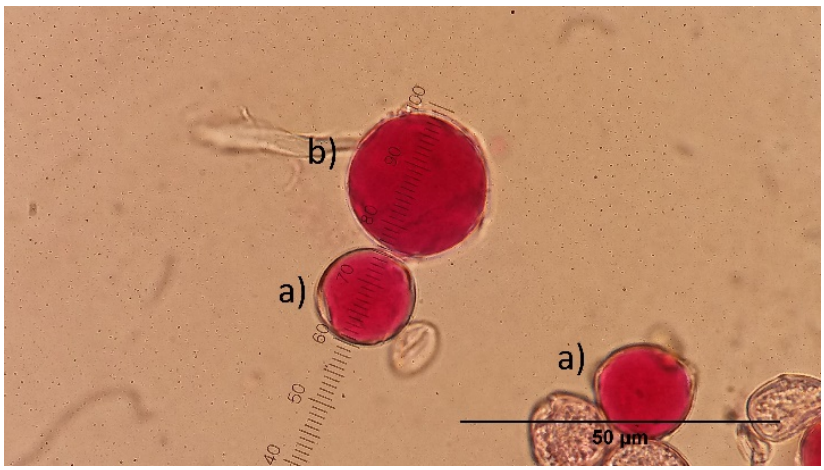
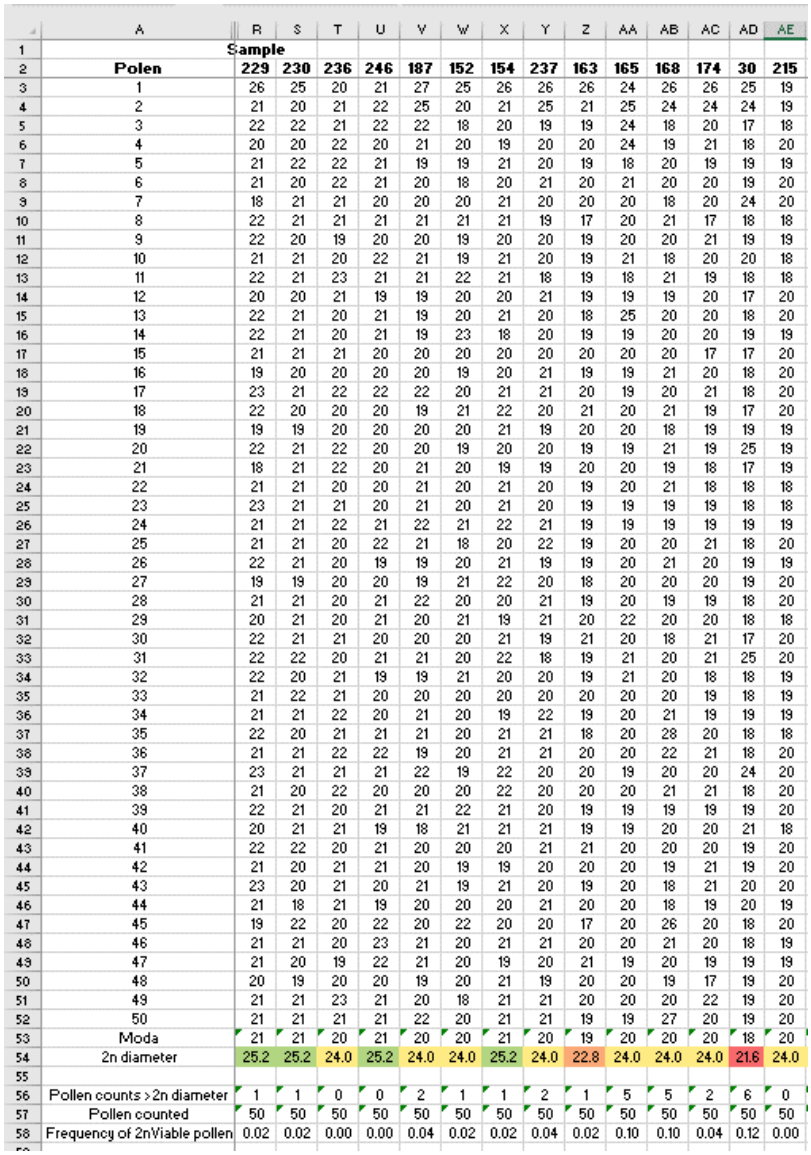


Figure 8. Example of a completed data grid with the pollen diameter of different diploid samples. 2NPol is calculated by multiplying the frequency obtained at the bottom of the chart with the percentage of pollen viability.



	A	B	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE
1		Sample													
2	Polen	229	230	236	246	187	152	154	237	163	165	168	174	30	215
3	1	26	25	20	21	27	25	26	26	26	24	26	26	25	19
4	2	21	20	21	22	25	20	21	25	21	25	24	24	24	19
5	3	22	22	21	22	22	18	20	19	19	24	18	20	17	18
6	4	20	20	22	20	21	20	19	20	20	24	19	21	18	20
7	5	21	22	22	21	19	19	21	20	19	18	20	19	19	19
8	6	21	20	22	21	20	18	20	21	20	21	20	20	19	20
9	7	18	21	21	20	20	20	21	20	20	20	18	20	24	20
10	8	22	21	21	21	21	21	21	19	17	20	21	17	18	18
11	9	22	20	19	20	20	19	20	20	19	20	20	21	19	19
12	10	21	21	20	22	21	19	21	20	19	21	18	20	20	18
13	11	22	21	23	21	21	22	21	18	19	18	21	19	18	18
14	12	20	20	21	19	19	20	20	21	19	19	19	20	17	20
15	13	22	21	20	21	19	20	21	20	18	25	20	20	18	20
16	14	22	21	20	21	19	23	18	20	19	19	20	20	19	19
17	15	21	21	21	20	20	20	20	20	20	20	20	17	17	20
18	16	19	20	20	20	20	19	20	21	19	19	21	20	18	20
19	17	23	21	22	22	22	20	21	21	20	19	20	21	18	20
20	18	22	20	20	20	19	21	22	20	21	20	21	19	17	20
21	19	19	19	20	20	20	20	21	19	20	20	18	19	19	19
22	20	22	21	22	20	20	19	20	20	19	19	21	19	25	19
23	21	18	21	22	20	21	20	19	19	20	20	19	18	17	19
24	22	21	21	20	20	21	20	21	20	19	20	21	18	18	18
25	23	23	21	21	20	21	20	21	20	19	19	19	19	18	18
26	24	21	21	22	21	22	21	22	21	19	19	19	19	19	19
27	25	21	21	20	22	21	18	20	22	19	20	20	21	18	20
28	26	22	21	20	19	19	20	21	19	19	20	21	20	19	19
29	27	19	19	20	20	19	21	22	20	18	20	20	20	19	20
30	28	21	21	20	21	22	20	20	21	19	20	19	19	18	20
31	29	20	21	20	21	20	21	19	21	20	22	20	20	18	18
32	30	22	21	21	20	20	20	21	19	21	20	18	21	17	20
33	31	22	22	20	21	21	20	22	18	19	21	20	21	25	20
34	32	22	20	21	19	19	21	20	20	19	21	20	18	18	19
35	33	21	22	21	20	20	20	20	20	20	20	20	19	18	19
36	34	21	21	22	20	21	20	19	22	19	20	21	19	19	19
37	35	22	20	21	21	21	20	21	21	18	20	28	20	18	18
38	36	21	21	22	22	19	20	21	21	20	20	22	21	18	20
39	37	23	21	21	21	22	19	22	20	20	19	20	20	24	20
40	38	21	20	22	20	20	20	22	20	20	20	21	21	18	20
41	39	22	21	20	21	21	22	21	20	19	19	19	19	19	20
42	40	20	21	21	19	18	21	21	21	19	19	20	20	21	18
43	41	22	22	20	21	20	20	20	21	21	20	20	20	19	20
44	42	21	20	21	21	20	19	19	20	20	20	19	21	19	20
45	43	23	20	21	20	21	19	21	20	19	20	18	21	20	20
46	44	21	18	21	19	20	20	20	21	20	20	18	19	20	19
47	45	19	22	20	22	20	22	20	20	17	20	26	20	18	20
48	46	21	21	20	23	21	20	21	21	20	20	21	20	18	19
49	47	21	20	19	22	21	20	19	20	21	19	20	19	19	19
50	48	20	19	20	20	19	20	21	19	20	20	19	17	19	20
51	49	21	21	23	21	20	18	21	21	20	20	20	22	19	20
52	50	21	21	21	21	22	20	21	21	19	19	27	20	19	20
53	Moda	21	21	20	21	20	20	21	20	19	20	20	20	18	20
54	2n diameter	25.2	25.2	24.0	25.2	24.0	24.0	25.2	24.0	22.8	24.0	24.0	24.0	21.6	24.0
55															
56	Pollen counts > 2n diameter	1	1	0	0	2	1	1	2	1	5	5	2	6	0
57	Pollen counted	50	50	50	50	50	50	50	50	50	50	50	50	50	50
58	Frequency of 2nViable pollen	0.02	0.02	0.00	0.00	0.04	0.02	0.02	0.04	0.02	0.10	0.10	0.04	0.12	0.00

After calculating the percentage of viable 2n pollen, you can rate its production according to Table 6.

Table 6. Categories to rate 2n pollen production.

Category	Viability range (%)	Description
0	0	Absent
1	0 < % ≤ 1	Low
3	1 < % ≤ 5	Moderate
5	% > 5	High

Source: Ordoñez et al. (2017)

4.7 Self-compatibility (SC)

Pollinations for selfing are performed manually with moderate-to-highly viable pollen, fresh or stored from the same campaign. If stored pollen will be used, careful validation of the identity of the genotypes must be performed during the entire process.

To test if a plant is self-compatible (SC) or self-incompatible (SI), a minimum of ten flowers (2-3 inflorescences) should be pollinated with its own pollen. Label each inflorescence with the genotype's identity, the number of flowers pollinated, and the date of selfing. Software such as CIPCROSS[1] or Intercross can help produce and print the labels (Courtney and Nielsen, 2019).

To maximize the number of fertilized ovules and seeds, it is common practice to perform three pollinations to the same flower so the entire receptive period of the ovules is profited. While doing this evaluation, it is important to pollinate all the flowers under evaluation the same number of times.

Observe the selfed inflorescences over the following days. If the flower or the pistil wilts, dries out, and/or falls – this is an indication of SI. If the base of the pistil starts swelling, this is an indicator of berry development and possible self-compatibility (Figure 9c and 9d).

The final indicator of self-compatibility is the production of seeds. Ripe berries can be harvested after two months. Seeds can be extracted by crushing the berries in a receptacle containing water and then draining the contents through mesh so that seeds are retained. Then, place the seeds on a paper towel and leave them to dry completely before counting and storing them (Ordoñez and Orrillo, 2012).

[1] Also see: https://research.cip.cgiar.org/gtdms/cipcross/

Figure 9. a) self-pollination; b) successful pollination: the bottom of the pistil has started swelling; c) three unsuccessful pollinations, the flowers have dried and fallen off; and three berries in early development; d) mature berries in the back a wilted flower from an unsuccessful pollination.

The following variables are useful for quantifying the selfing efficiency:

4.7.1 Fruit set percentage (FSP):

This value indicates the percentage of pollinations that ended in fruit development. Fruit development is an indicator of an absence of pre-zygotic reproductive barriers such as gametophytic self-incompatibility mediated by S-RNase alleles.

$$FSP = \frac{Number\ of\ total\ fruits\ per\ plant \times 100}{Number\ of\ total\ flowers\ pollinated}$$

4.7.2 Seed set (SS):

The average of this value indicates the number of seeds obtained per fruit for a given genotype. Values above 200 seeds per fruit indicate high compatibility.

$$SS = \frac{Total\ number\ of\ seeds}{Total\ number\ of\ fruits}$$

In cases of SI, when using viable pollen, it is advisable to perform a test for in-vivo pollen tube growth and observe if the pollen tubes are reaching the ovaries. For this technique, pollinated pistils need to be collected 24-48 hours after pollination in Farmer's fixative and then stained in an aniline blue-based solution for observation under UV light through a fluorescence microscope (Ordoñez et al. 2017).

5 Complementary Variables

These variables are not within the group of reproductive biology or cytology. Nevertheless, they are useful when selecting the best clones.

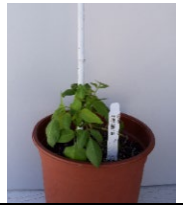
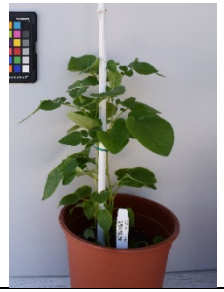
5.1 Plant vigor (PlVig)

This evaluation is usually measured once at 45 DAP. There are different protocols for plot evaluations in field experiments (De Haan et al., 2014, UW-Madison Russell Lab, 2013, Castillo et al., 2016). In our adaptation, we will follow the same categories, but we will apply them to an individual plant instead of a plot. The evaluation will be taken twice at 45 DAT and 75 DAT considering that, within a progeny, not all plants develop at the same rate. Table 7 provides description of the categories and reference pictures. It is important to note that this a subjective evaluation and the photos are merely for reference. They do not have to be a perfect match. It is good practice during evaluation to walk around all the material and identify the weakest and strongest plants (to calibrate the eye) and then perform the evaluation of other plants. It is also advised that the same person perform both evaluations (45 and 75 DAT).

5.2 Type of plant (PlTyp)

This trait is recorded for characterizing plants and tracing parental phenotypes (referentially). It is based on noting the difference in leaf size between Tuberosum and Andigena. Leaf size will be wider for Tuberosum genotypes (Hosaka et al., 2018). These ratings are organized in four categories according to the phenotype of the plant (Table 8). This evaluation is measured once at 60 DAT.

Table 7. Categories for evaluating plant vigor, reference photos and descriptions.

Categories and description for plant vigor				
1	3	5	7	9*
				
Very weak plant Less than 20 cm height. Few stems and few leaves. Leaves can be light green or very pale. Slow growth.	Weak plant 20-30 cm height Thin stems and few leaves Leaves can be light green or very pale. Slow growth.	Average plant One or more stems. Normal growth.	Moderately strong plant 50 cm height One or more thick stems Vigorous growth	Strong plant 70 cm height One or more thick stems Dark green-colored leaves Rapid vigorous growth

*Category 9 is usually seen in tetraploid advanced cultivars.

Table 8. Categories for evaluating potato plant type phenotype.

Categories and description for type of plant			
1: Tuberosum	2: Mixed	3: Andigena	4: Wild
			
Wide leaflets Shorter plants	Phenotypes with characteristics from Tuberosum and Andigena.	Narrow and numerous leaflets Pigmentation on stems and abaxial veins from the leaflets is common. Taller plants	Phenotypes that do not coincide with the previous three.

6 Analysis

After collecting data, cleaning them, and arranging the fieldbook as shown in Figure 6, the data is ready for descriptive analysis, analysis of variance (ANOVA) and comparisons tests. Figure 10 provides a sample database but does not contain pollen traits because they are taken only once during the evaluation. Thus, pollen data will be analyzed separately. The idea is to obtain a final document with mean values for all genotypes.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	ORD	CIPF	CIPN	Breeder Code	REP	Fe. CIPN	Fe. Breeder Code	Ma. CIPN	Ma. Breeder Code	DAT	Flwdgr	Lsty	PlVig	CFL	ICFL	PlTyp
2	1	CIP316330	CIP316330.054	DHP-330.054	R1	CIP388676.1	Y84.027	CIP800951	IVP-35	45	1	3	5	6	3	
3	2	CIP316330	CIP316330.054	DHP-330.054	R2	CIP388676.1	Y84.027	CIP800951	IVP-35	45	1		3			
4	3	CIP316330	CIP316330.060	DHP-330.060	R1	CIP388676.1	Y84.027	CIP800951	IVP-35	45	0		1			
5	4	CIP316330	CIP316330.060	DHP-330.060	R2	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
6	5	CIP316330	CIP316330.065	DHP-330.065	R1	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
7	6	CIP316330	CIP316330.065	DHP-330.065	R2	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
8	7	CIP316330	CIP316330.082	DHP-330.082	R1	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
9	8	CIP316330	CIP316330.082	DHP-330.082	R2	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
10	9	CIP316330	CIP316330.083	DHP-330.083	R1	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
11	10	CIP316330	CIP316330.083	DHP-330.083	R2	CIP388676.1	Y84.027	CIP800951	IVP-35	45						
1494	169	CIP500007	CIP500007.16	C00LG5-07.16	R2	CIP720045	ATZIMBA	CIP800951	IVP-35	105	1		5			1
1495	170	CIP597013	CIP597013.7	C97HT-13.7	R1	CIP800265	KUFRI SINDHURI	CIP801037	IVP-101	105	0		3			1
1496	171	CIP597013	CIP597013.7	C97HT-13.7	R2	CIP800265	KUFRI SINDHURI	CIP801037	IVP-101	105	0		3			1
1497	172	CIP597043	CIP597043.13	C79HT-43.13	R1	CIP720088	ACHIRANA	CIP800951	IVP-35	105	0		7			1
1498	173	CIP597043	CIP597043.13	C79HT-43.13	R2	CIP720088	ACHIRANA	CIP800951	IVP-35	105	1		7			1
1499	174	CIP597043	CIP597043.24	C97HT-43.13	R1	CIP720088	ACHIRANA	CIP800951	IVP-35	105	1		5			1
1500	175	CIP597043	CIP597043.24	C97HT-43.13	R2	CIP720088	ACHIRANA	CIP800951	IVP-35	105	1		5			1
1501	176	CIP598064	CIP598064.3	C98HT-64.3	R1	C95T-07.3 x	C95T-07.3 x	CIP801037	IVP-101	105	3	3	3	1	2	1
1502	177	CIP598064	CIP598064.3	C98HT-64.3	R2	C95T-07.3 x	C95T-07.3 x	CIP801037	IVP-101	105	0		3			1
1503	178	CIP598156	CIP598156.14	C98HT-156.14	R1	CIP800048	800048=(DESIREE)	CIP801037	IVP-101	105	0		3			1
1504	179	CIP598156	CIP598156.14	C98HT-156.14	R2	CIP800048	800048=(DESIREE)	CIP801037	IVP-101	105	0		3			1
1505	180	CIP598158	CIP598158.9	C98HT-158.9	R1	CIP800959	Granola	CIP801037	IVP-101	105	0		3			1
1506	181	CIP598158	CIP598158.9	C98HT-158.9	R2	CIP800959	Granola	CIP801037	IVP-101	105	1		3			1
1507	182	CIP598159	CIP598159.59	C98HT-159.59	R1	CIP573079	I-1035	CIP801037	IVP-101	105	1		5			1
1508	183	CIP598159	CIP598159.59	C98HT-159.59	R2	CIP573079	I-1035	CIP801037	IVP-101	105	1		5			1

Fieldbook

Figure 10. Excel view of a fieldbook of evaluated reproductive characteristics. It is crucial to consider the sample's pedigree ORD: order, CIPN: CIP Number, REP: repetition, Fe.: Female, Ma.: Male.

A calculated and useful trait is flowering peak (FlwPk), which can give us information about the earliness of the genotype. To calculate it on RStudio, use the following code:

```
ddd <- read.csv("Data.csv") # “Data.csv”would be the above table.

out <- ddd[1, ]

for (i in 1: length(ddd$CIPN)) {

temp <- ddd[ddd$ORD == i, ] #ORD is the unique identifier as seen
on column A in Figure 10.
```

```
x <- which.max(temp$Flwdgr)

out <- rbind(out, temp[x, ])

}

out <- out[-1, ]

write.csv(out, 'Peak Flowering.csv')
```

Depending on the breeder objectives, an advisable cut-off value for Flwdgr may be in the range of 3.5 to 7. Depending on the QPol, PollV, and 2NPol, the selected genotypes will be classified as potential males or females. For example, if a genotype has an average Flwdgr of 7 and its pollen viability is below 60% or male sterile, it would be classified as potential female. A potential male genotype must have pollen viability above 60% and a QPol greater than 3. The word “potential” is used here because crossability between two genotypes involves considering other hybridization barriers such as EBN, cytoplasmic male sterility, or gametophityc self incompatibility (S-alleles). And, of course, other factors may include the breeding value related to the targeted trait and their general and specific combining abilities (Ortiz and Golmirzaie, 2004).

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